Peripheral Detection of S100β During Cardiothoracic Surgery: What Are We Really Measuring?

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Background. **S100β has been used in cardiac surgery to identify patients with postoperative neurologic complications. However, extracranial proteins may falsely elevate measurements of serum S100β;. Objectives of this study were (1) to quantify S100β levels in serum and pericardial cavity during coronary artery bypass grafting (CABG), and (2) to identify proteins recognized by standard immunodetection as S100β.**

Methods. **Systemic and pericardial cavity blood from 5 patients undergoing CABG were sampled before, during, and after cardiopulmonary bypass (CPB). A commercially available enzyme-linked immunosorbent assay (ELISA) kit was used to quantify S100β. Two-dimensional gel electrophoresis, Western blot, and mass spectroscopy were also performed to identify S100â and other proteins.**

Results. **Mean S100β levels measured by ELISA, systemic and pericardial cavity blood were (in ng · mL^{-1}) 1.0 ± 0.46 and 111 ± 71 before CPB, 0.6 ± 0.11 and 113 ± 54 during CPB, and 1.7 ± 0.64 and 101 ± 42 after CPB, respectively. However, gel electrophoresis and Western blot analysis revealed proteins other than S100β to be present in the pericardial cavity giving a falsely elevated serum S100â levels measured by immunoassay. Mass spectroscopy of identified potential candidates revealed contaminants including haptoglobin I precursor, apolipoprotein A-1 precursor, complement factor B precursor, and complement C3 precursor.**

Conclusions. **S100β immunoassays are not specific for S100â and give a falsely elevated reading due to contaminants from the surgical field that cross react with the assay's antibody. This does not appear to be an issue in nonsurgical patients. Caution must be exerted when evaluating immunodetection results for low-abundance proteins under conditions where contamination of the sample is likely.**

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Detection of peripheral serum markers can be a reliable tool in the determination of ongoing or past brain damage [1, 2]. Serum S100β concentration has been measured following head injury, cardiac surgery, and brain tumor where blood-brain barrier (BBB) integrity and(or) brain damage have been suspected [1–8]. Several issues arise when detecting protein markers in peripheral blood, including the specificity of the test used; proteins are commonly assessed by immunodetection. Since antibody specificity is far from absolute, caution must be used when interpreting these results. For S100β, effort has been devoted to the selection of antibodies that specifically recognize S100β and not other family members [9]. However, the overall lack of cross reactivity with other serum proteins has not yet been determined.

Specificity of the cellular release site and timing of a marker's appearance are also important issues. To be clinically useful, serum levels of the putative marker must closely correlate with a well-defined pathologic condition and be released by cells that are associated with the pathologic sequelae. In the case of S100β, conflicting results from cardiac surgery have suggested that either the site of release of these markers is not fully understood or that the tests used may have intrinsic specificity problems [5]. In fact, (1) levels of S100β in serum are influenced by manipulations of peripheral hemodynamics by surgical procedures, such as in cardiac surgery, that do not necessarily affect the brain, and (2) the levels observed after cardiac surgery are comparable to those observed with serious brain damage [5, 10].

S100β can also be used as a marker of BBB leakage [2, 6]. In the central nervous system (CNS), the function of S100β and its cellular distribution are fairly well understood [11]. S100β is located primarily in astrocytes and can be released to the perivascular space and extravasate immediately after BBB opening. Very low levels compared to the brain were found in non-CNS tissues [5, 11–14]. Clinical studies have shown that steady state levels of S100β in serum following maximal opening of the BBB reach the theoretical plateau of 0.34 ng · mL^{-1} [6]. Thus, serum values that exceed this threshold may imply an

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Abbreviations and Acronyms

ANOVA = analysis of variance
APOA-1 = apolipoprotein A-1
BBB = blood-brain barrier
CABG = coronary artery bypass graft
CNS = central nervous system
CPB = cardiopulmonary bypass
ELISA = enzyme-linked immunosorbent assay
HDL = high density lipoprotein
kDa = kiloDaltons
MW = molecular weight
PAGE = polyacrylamide gel electrophoresis
SDS = sodium dodecylsulfate
SEM = standard error of mean

ongoing process of brain damage or accumulation of S100β from nonbrain sources [6].

As mentioned above, the most exorbitant increases in S100β blood levels have been observed during cardiopulmonary bypass (CPB) procedure [5, 10, 15], where S100β has been used, although with very limited success, to monitor the possibility of ongoing brain injury. While the procedure itself does not commonly lead to permanent brain damage, S100β values measured in the blood of CPB patients were comparable to the serum levels found after lethal head injury [16]. It was concluded that sources other than the CNS may contribute to these abnormally elevated values or that S100β can be released from the brain at levels previously undocumented. Given that the S100β serum levels obtained during CPB were significantly elevated compared to what was found experimentally and estimated theoretically, we tested the hypothesis that elevated S100β levels during surgical procedures were due to inherent pitfalls in the testing strategy used. We investigated (1) the possibility that sources other than astrocytes can contribute to S100β blood levels during CPB, and (2) the possibility that a contaminating agent(s) may give exaggerated S100β serum values after CPB procedure.

Material and Methods

Patients and Surgical Procedure

Informed consent was obtained from five patients scheduled for elective coronary artery bypass (CAB) surgery, and the study was approved by the Institutional Review Board of The Cleveland Clinic Foundation. None of the patients had any history of cerebral disease or renal impairment and postoperatively there was no clinical evidence of neurologic adverse outcome.

Anesthesia was induced with a combination of midazolam 3 to 5 mg (Abbott Laboratories, North Chicago, IL), fentanyl (Abbott Laboratories, North Chicago, IL) up to 0.25 mg, and thiopental 3 to 5 mg $\cdot$ kg^{-1} (Abbott Laboratories, North Chicago, IL), intravenously. It was subsequently maintained using inhaled isoflurane 1% to 2% (Abbott Laboratories, North Chicago, IL) supplemented with fentanyl up to a total of 1 mg. Muscle relaxation was achieved with pancuronium (GensiaSicor Pharmaceutical, Irvine, CA) up to a total of 10 mg. Cardiopulmonary bypass was instituted utilizing a conventional modular roller pump system (Sarns 8000 Terumo Cardiovascular, Ann Arbor, MI) and polyvinyl chloride tubing (Jostra, Anaheim, CA). The circuit also included a 25 μm arterial filter (Jostra, Anaheim, CA), adult membrane oxygenator (Jostra, Anaheim, CA), and an integrated, hardshell, venous-cardiotomy reservoir (Jostra, Anaheim, CA). The entire circuit was heparin coated. The circuit was primed with approximately 1,500 mL of Plasma-Lyte A (Baxter Healthcare Corp., Deerfield, IL), 250 mL 20% mannitol (Baxter Healthcare Corp., Deerfield, IL), 10,000 U porcine-intestinal heparin (Elkins-Sinn, Inc., Cherry Hill, NJ), and 15 meq sodium bicarbonate (Abbott Laboratories, North Chicago, IL). Operations were performed through a median sternotomy. Standard cannulation for CPB was performed with ascending aorta cannulation and dual-stage cannulation of the right atrium, after administration of 300 U $\cdot$ kg^{-1} of heparin. Cardiolegia cannulas were placed both antegrade and retrograde. Once on CPB, the arterial flow was adjusted to 2.0 to 2.4 L $\cdot$ min^{-1} $\cdot$ m^{-2}, and blood pressure was maintained between 50 and 70 mm Hg, with administration of phenylephrine (Abbott Laboratories, North Chicago, IL) if required. The aorta was cross clamped and cold blood cardioplegia (Buckberg solution) was administered either antegrade or retrograde or in combination every fifteen minutes and was delivered at a ratio of four parts blood to one part crystalloid (4:1). Body temperature was either kept normothermic or allowed to drift to 34°C during CPB. After the bypass grafts were placed, patients were weaned from CPB and decannulated and heparin was reversed with protamine (American Pharmaceutical Partners, Schaumburg, IL). Blood samples, approximately 5 mL/draw, were collected before, during, and after CPB from the systemic circulation and pericardial cavity.

Immunoassay Determination

The LIA-mat Sangtec 100 kit (Bromma, Sweden) was used to quantitate S100β. The kit uses a monoclonal two-site immunoluminometric methodology (sandwich principle). The assay discriminates between the A1-subunit and B-subunit of the S100 protein through the use of three patented monoclonal antibodies (SMST 12, SMSK 25, and SMSK 28) and the sensitivity is 0.01 ng $\cdot$ mL^{-1}.

Protein Filtration

One milliliter of sample was pipetted into a centrifugal filter unit (50 kDa) and centrifuged at 7,500xg using a swinging bucket rotor (Beckman Instruments, Palo Alto, CA) for approximately 20 to 30 minutes. If necessary, centrifugation time was increased until equal volumes were achieved in both the upper and lower chambers of the centrifugal filter unit (0.5 mL each).

Gel Electrophoresis

Sodium dodecylsulfate (SDS) and non-SDS polyacrylamide gel electrophoresis (PAGE) were performed by standard methods [17]. In non-SDS PAGE samples were analyzed in nondenaturing conditions. To address this, denaturating agents were excluded from the sample buffer and the running buffer and during the preparation of the gel. Fifty milliliters of sample buffer (5×) were obtained by mixing 15.5 mL of 1 mol/L tris-hydrochloric acid pH 6.8, 9.5 mL of water, 25 mL of glycerol, and a trace amount of bromophenol blue. An electrophoresis buffer (1×) was obtained by dissolving 3 g of tris-hydrochloric acid and 14.4 g of glycine in 1 L of deionized water and adjusting to a final pH of 8.3.

Western Blot and Two-Dimesional Gel Electrophoresis

Identification of S100β protein was performed by Western blotting techniques as described elsewhere [7]. Two-dimensional gel electrophoresis was performed according to the methods reported in our previous work [7]. Two different primary antibodies to S100β were used: sheep antibovine (1:1,000, QED Bioscience Inc.) and mouse antibovine (1:1,000, Sangtec; Bromma, Sweden). After washes, the transfer membrane was incubated with secondary horseradish peroxidase: antisheep IgG (rabbit) (1:5,000, Calbiochem, LA Jolla, CA) or antimouse IgG (rabbit) (1:5,000, Dako Corporation, Carpinteria, CA) for 2 hrs. Specific blots were visualized by enhanced chemiluminescence reagent (ECL plus, Amersham Pharmacia Biotech, UK).

Mass-Spectroscopy

We used a LC-MS system Finnigan LCQ-Deca ion trap mass spectrometer system with a Protana microelectrospray ion source interfaced to a self-packed 10 cm × 75 um id Phenomenex Jupiter C18 reversed-phase capillary chromatography column. Data were analyzed by using all CID spectra collected in the experiment to search the National Center of Biotechnology Information (NCBI) nonredundant database with the search program TurboSequest. All matching spectra were verified by manual interpretation. The interpretation process was also aided by using the programs Mascot and Fasta to perform additional searches, as needed.

Statistical Methods

Data are presented as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) was used to determine significance. Origin 7.0 (Microcal) was used for statistical analysis.

Results

Thirteen samples from systemic blood and 15 from the pericardial cavity were obtained. Serum S100β levels before surgery were within normal levels (<0.12 $ng \cdot L^{-1}$). Significant differences between the two sets of samples were found at any given time point (S100β levels determined by enzyme-linked immunosorbent assay [ELISA]; Fig 1). On average, pericardial cavity S100β levels were elevated compared to peripheral blood.

The molecular weight (MW) of S100β is approximately 11 kDa. We hypothesized that the presence of high MW contaminants in the more than 50 kDa MW fraction was one of the causes of exorbitant S100β levels in the cavity samples. All samples were filtered through a 50 kDa MW cutoff filter, and remeasured by ELISA. As predicted by our hypothesis, high MW fractions gave an elevated signal compared to the less than 50 kDa MW fraction where S100β is expected (Fig 1, panels *A* and *B*). The low MW fraction obtained from venous samples gave S100β levels only slightly above normal values (0.17 $ng \cdot mL^{-1}$; Fig 1, panels *A* and *C*). In contrast, the concentration of S100β in the low MW cavity sample was significantly elevated above normal levels (22.4 $ng \cdot mL^{-1}$; Fig 1, panels *B* and *D*).

The low MW fraction displayed no significant changes in systemic blood, while the high MW fraction gave increasingly high values up to a final value of 1.7 ± 0.6 $ng \cdot mL^{-1}$ (Fig 1, panel *C*).Cavity samples produced a highly elevated signal, as measured by ELISA, during the time of the CPB in the more than 50 kDa fraction (113 ± 54 $ng \cdot mL^{-1}$) compared to the less than 50 kDa fraction (25 ± 6 $ng \cdot mL^{-1}$, Fig 1, panel *D*),suggesting that regardless the nature of the signal measured by ELISA, the cavity region produced the greatest quantity of immunosignal. Taken together, these results suggested that a high MW molecule, other than S100β, may appear in the systemic circulation after recirculation of the cavity blood contents.

Probing further with the Western blot technique under nondenaturating conditions, a high MW band appeared in all the samples: the *dashed arrow* in Figure 2 refers to nonspecific binding found in all samples, while the *continuous arrow* shows an additional, well-defined band that was stronger in the cavity (indicated by *C*) samples throughout the procedure. To quantify the patterns of S100β release under both conditions, we measured S100β in the same samples by ELISA; the values obtained are shown at the bottom of Figure 2. Note that there was a consistent dissociation of cavity versus peripheral (indicated by *P* blood readings, the former giving much higher values. While elevated S100β values measured by ELISA correlated with the presence of high MW bands, signals attributable to S100β proper on the gel remained largely unchanged throughout the experiment (see low MW bands corresponding to the S100β standard loaded for comparison). Significant increases in the amount of measurable S100β proper were evident only in serum samples taken hours after the surgery (*asterisks*, Fig 2).

Western blot analysis of the high MW venous fraction conducted under denaturing conditions using the same primary antibody included in the ELISA kit, displayed a distinct double band around 90 kDa, with an additional single band between 35 and 40 kDa. This provided information about the MW of the hypothetical contami-

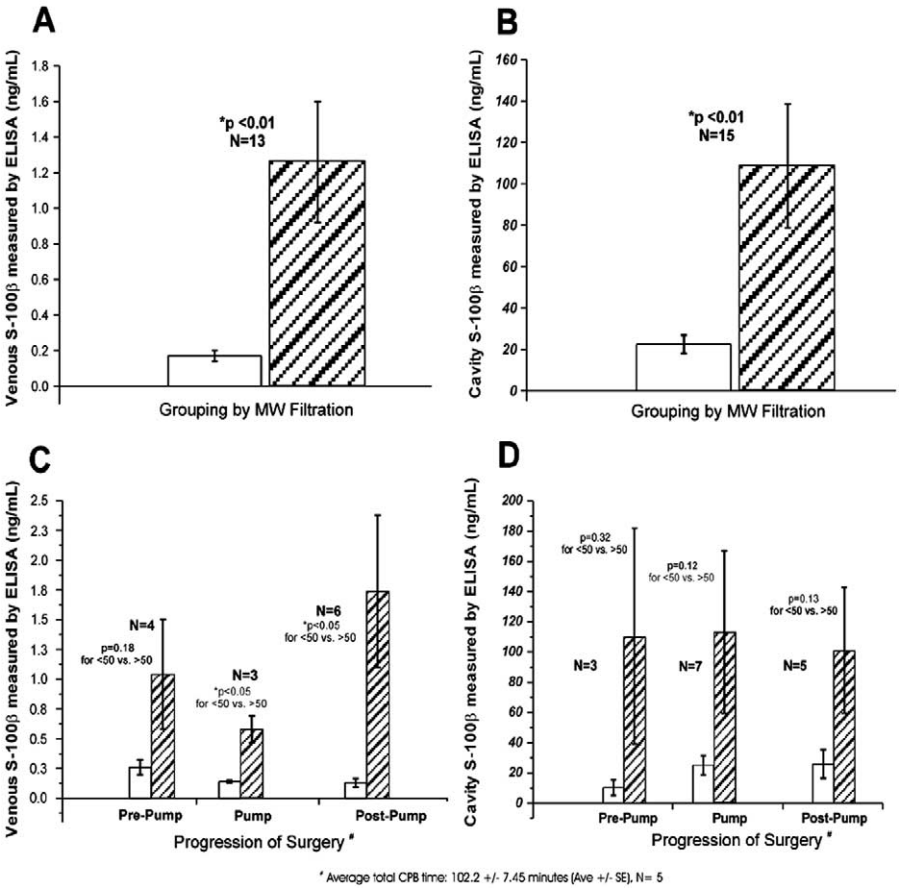

Fig 1. (A and B) The significant difference (p < 0.01) between the S100β *measured in fractionated samples (*MW *<50* kDa *and* MW *>50* kDa*) from both venous (A) and cavity (B) samples obtained during* CPB *surgery. The S100β values are expressed in mass units (*ng · mL^{-1}*) in order to emphasize the relative quantity of protein measured by ELISA. (C and D) The relative difference of* S100β *measured in fractionated samples during 3 time points during CPB surgery (prepump, pump, and postpump) for both venous (C) and cavity (D) samples. Note that a significant difference in* S100β *was only measured in serum (venous) samples at pump and postpump time points (p < 0.05). (Note different axes ranges in A and B).* White bar = *<50 kDa;* hatched bar = *>50 kDa.* (Ave = *average;* CPB = *cardiopulmonary bypass;* ELISA = *enzyme linked immunosorbent assay;* kDa = *kiloDaltons;* min = *minutes;* MW = *molecular weight;* SE = *standard error;* vs = *versus.)*

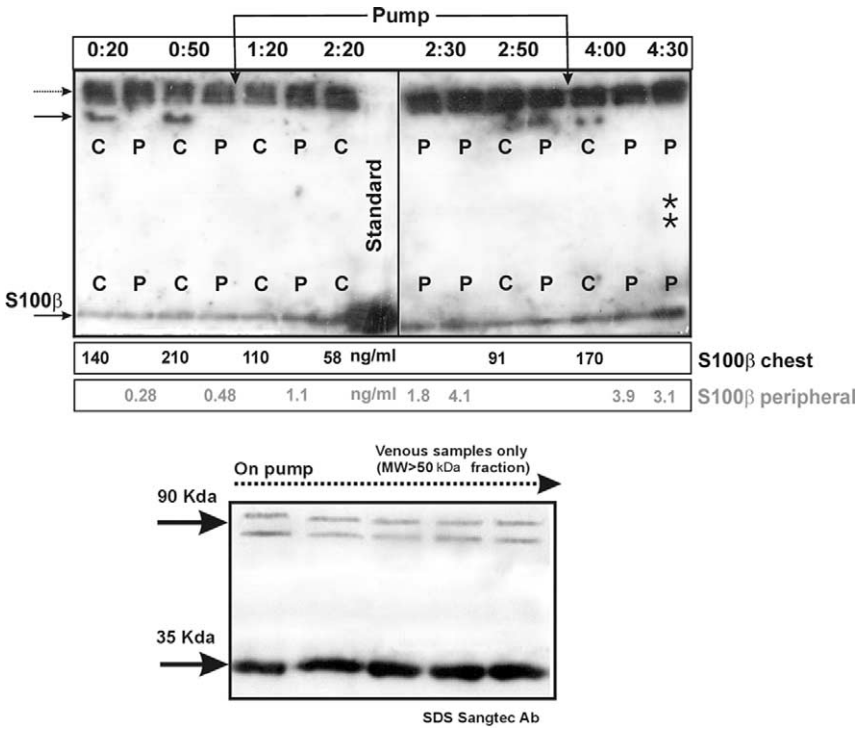

Fig 2. (Top) Western blot analysis (nondenatured preparation) of cavity and peripheral samples during CPB surgery. Values at the bottom *indicate the relative concentrations of S100β as measured by ELISA. (Bottom) A second Western blot analysis of high MW (MW >50* kDa*) venous fraction (peripheral) conducted under denaturing conditions utilizing the same S100β primary antibody utilized to quantitate the protein with the commercially available ELISA kit. (*C = *cavity sample; CPB = cardiopulmonary bypass; ELISA = enzyme linked immunosorbent assay;* kDa = *kiloDaltons;* MW = *molecular weight;* P = *peripheral sample.)*

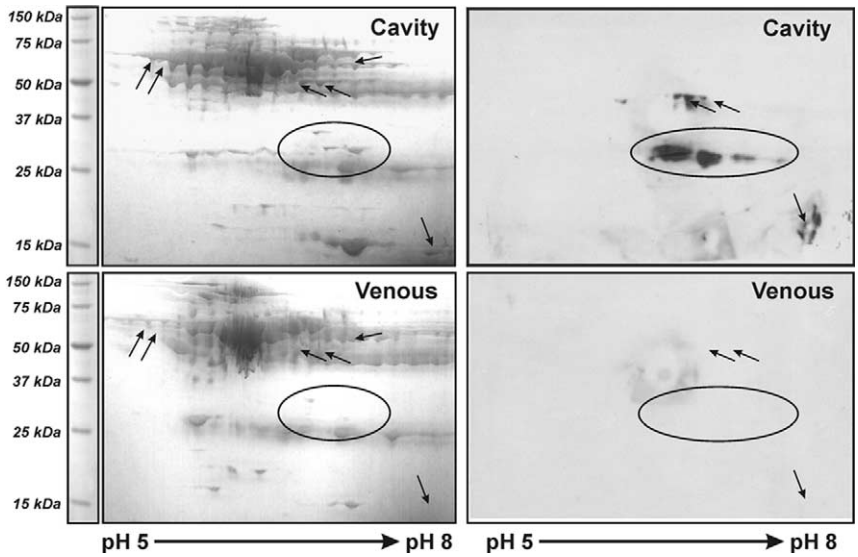

Fig 3. Left panels represent comparative 2D-PAGE scans of samples from cavity (top) and venous (bottom) collection during CPB surgery using Coomassie blue, total protein stain. The right panels display the same samples analyzed by Western blot technique for S100β protein (using Sangtec 100 kit antibodies). The circled and arrow markings *indicate corresponding areas of varied protein detection. (*2D-PAGE = *two-dimensional polyacrylamide gel electrophoresis; CPB = cardiopulmonary bypass.)*

nants and showed clearly how this antibody can recognize a protein other than its desired target. To further investigate the nature of these possible contaminants, systemic and cavity protein samples were separated by bidimensional gel electrophoresis (Fig 3). Qualitatively, identical results were obtained in all samples tested (n = 5).

Additional experiments were performed to identify the molecular nature of the contaminants (Fig 3, right panel). In fact, the possibility exists that this apparent antibody cross reactivity was due to detection of S100β to other serum proteins, altering the signal's MW and isoelectric profile. Note that large quantities of high MW proteins were immunopositive in the chest cavity sample. No immunosignal was detected under these loading conditions in the venous blood sample.

Mass spectroscopy identification of the high MW polypeptide fragments led to a short list of eight potential candidates but only four of these were repetitively detected in the cavity fluid (Table 1). These proteins represent the possible contaminants affecting the S100β determination during CPB.

Comment

Unequivocal quantitative and qualitative protein identification represents the most crucial step whenever one wishes to link the presence of a serum protein marker to organ dysfunction or pathology. Specificity of source, time of appearance in the circulation, blood level, and appropriate identification tools are the keys for an exact determination. The aim of this work was to understand possible confounding factors affecting the evaluation of S100β in blood of patients undergoing the CPB procedure. In particular, we investigated the possibility that sources other than astrocytes (eg, tissue surrounding the chest cavity) may contribute to S100β blood levels during CPB as well as the concept that a contaminating agent may give exaggerated S100β serum values during this procedure. Our results show that the most likely cause of the exorbitant serum S100β values observed during and after cardiothoracic surgery is attributable to cross reactivity of the detection system with proteins present in the surgical field. This was demonstrated by three independent strategies discussed below.

Table 1. Molecular Nature of the Protein Detected by Mass Spectroscopy in Cavity Samples

Haptoglobin I precursor	NCBI #P00737, MW 38.5 kDa pI
Apolipoprotein A-I precursor	NCBI #P02647, MW 30.8 kDa pI
Complement factor B precursor	NCBI #P00751, MW 85.5 kDa pI
Complement C3 precursor	NCBI #P01024, MW 187.2 kDa

MW = molecular weight; NCBI = National Center for Biotechnology.

First, we have shown that filtration of serum proteins from either peripheral blood or cavity fluids reveals a surprising amount of immunodetectable S100β in the high MW fraction. This was unexpected because (1) S100β is a low MW protein, and (2) the exceedingly high levels of S100β measured during CPB procedures were previously attributed to release of S100β by non-CNS sources (eg, bone marrow, fat tissue, etc) [5]. Our results do not completely rule out a contribution of extra-brain S100β to serum levels, but suggest that this confounding factor plays, at best, a small role. Whatever the nature of this abnormal signal, our results clearly demonstrated that its origin is indeed extracranial, and that dilution in serum occurs during extracorporeal circulation. This has been directly demonstrated by others using the astute strategy of comparing serum S100β levels under different hemodynamic and hemodilution conditions [5].

The second set of experiments demonstrated a further dichotomy between S100β detection by MW-independent approaches and protein electrophoresis (Figs 2 and 3). Thus, we were able to demonstrate that potentially cross-reacting proteins were present in the cavity samples. These were clearly distinct from S100β, as shown by direct comparisons with standards preloaded on the gel. In agreement with the experiments described

above, these signals were clearly predominant in the high MW range. While the results discussed so far strongly argued in favor of a significant lack of specificity of the MW-independent approach commonly used for diagnostic purposes, it was still possible that linkage of S100β to other proteins could have occurred.

The likelihood of protein-protein interaction is far from remote, in particular in body fluids. Perturbations of equilibria (pH, temperature, etc) have powerful disruptive effects on these delicate interactions. To rule out that this was not the cause underlying the observed differences between samples, we subjected bidimensional blots to proteomic analysis, an approach we previously used for similar purposes [7]. The data clearly demonstrated that the large immunosignals revealed by the anti-S100β antibodies were not attributable to S100β or other proteins of the S100 family. In fact, none of the sequences derived were matched to these peptides.

An important question is how our findings impact the interpretation of data obtained with immunodetection of S100β. The following section tries to address this issue, keeping in mind that serum S100β has been used to predict both blood-brain barrier leakage and(or) brain damage.

Significance of Serum S100β

A large corpus of clinical and experimental results have shown that serum S100β can be a sensitive peripheral marker of brain damage; historically, most of the work was done in patients undergoing cardiothoracic surgeries [2, 18]. It was generally assumed that the specific brain protein S100β is released systemically after cerebral lesion. More recently, this view was challenged and experiments have shown that cerebral lesions are not necessary to produce S100β elevation in serum. Thus, Kapural and colleagues [2] have shown that S100β levels can be indicators of BBB function even in the absence of neuroglial damage. It is thus not surprising that a quantitative relationship exists between serum S100β levels and specific cerebrovascular or parenchymal pathologies [6, 19]. In severe head injury [16, 20, 21] and stroke patients [22], S100β levels correlate with clinical outcome. For example, patients who died after head injury had significantly higher serum S100β values compared with those who survived (2.7 $\mu g \cdot L^{-1}$ vs 0.5 $\mu g \cdot L^{-1}$) [16, 20, 21].

If the values observed in our study were the result of S100β released in response to neuroglial dysfunction, then it would be difficult to accept serum values obtained after CPB procedures with nondetrimental neurologic outcome [5, 23]. Several explanations were offered to justify this discrepancy, including contamination by non-CNS sources during surgery [5]. Our results confirm the presence of contaminants, but these were non-S100β proteins presumably released by extra-CNS tissue.

Despite these results, the notion that S100β released by the brain into the peripheral circulation has diagnostic values remains valid. The presence of high MW cross reactants warrants the development of filtration steps before the immunodetection step. Alternatively, a different test (or antibodies) can be developed. The problems we unveiled, however, seem to be only present during surgical procedures. In fact, exorbitantly elevated S100β values have not been described in "intact" patients, including those affected by either neurologic disorders or brain tumors [6, 7, 14, 19]. Our findings could also explain the discrepancy between neurologic patients affected by trauma or trauma plus various bodily injuries [24, 25]. It is in fact likely that, in the latter population, proteins other than S100β were measured as a consequence of multiple traumatic injuries.

Significance and Sources of Contaminants

Our extensive search for non-S100β proteins responsible for spurious results led to a short list of candidates that were repetitively detected in the cavity fluids. Haptoglobin combines with free plasma hemoglobin to prevent the loss of iron through the kidneys. Apolipoprotein A-1 (APOA-1) precursor is the source of APOA-1, major components of plasma high density lipoprotein (HDL), which participates in the reverse transport of cholesterol from tissue to the liver for excretion by promoting cholesterol efflux. Interestingly, one of the precursor proteins that our proteomic analysis unveiled (APOA-1) was shown to be expressed in human brain, where it may correlate with cognitive function by direct or indirect HDL mechanisms [26]. Complement factor B and complement factor C3 precursors are linked to proinflammatory changes and are thus expected to increase in the surgical field. Their predominantly peripheral origin rules out CNS sources.

While filtering cavity samples at 50 kDa promptly removed the offending protein from immunodetection, mass spectroscopy analysis revealed molecular weights close to S100β. This apparent inconsistency can be easily explained by the fact that all these protein species readily assemble with abundant protein (eg, hemoglobin).

Conclusions

We demonstrated the limitations associated with the use of S100β for the evaluation of brain damage and patients' outcome during surgical procedures. Nonetheless, the currently available methods used to measure serum S100β are still valid for the evaluation of neurologic disorders associated with either BBB leakage or brain damage, provided significant extra-CNS sources of contaminants are not present. Filtering or immune-preabsorption of samples may be required to completely rule out cross contamination by other protein species.

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INVITED COMMENTARY

In this study by Fazio and colleagues, an important mystery in the field of peripheral marker detection of cardiac surgery-associated neurologic injury is answered. In an ideal world, the use of serum markers would provide an attractive means to diagnose brain injury in a setting where the clinical diagnosis is frequently hampered by the residual effects of anesthesia and imaging diagnostic techniques that either lack specificity and sensitivity or present logistical problems.

Indeed, a great deal of work has been published regarding the relationship of S100β to neurologic injury in this setting. S100β was once thought to be an ideal marker of cardiac surgery-associated neurologic injury because its glial cell origin made it brain specific. However, subsequent reports that there may be extra-cerebral sources for S100β led to the questioning of its specificity as a marker to identify cerebral injury in this setting [1]. This led to considerable dampening of enthusiasm for its continued investigation and also presented a perplexing problem. That is, if S100β was allegedly brain specific, why was it present in such high concentration in the blood present in the cardiotomy return?

This present study addresses the reasons responsible for this apparent extra-cerebral S100β. What the authors now demonstrate is that the S100β immunodetection techniques that previously identified S100β in high concentration in the blood from the pericardial cavity (that was returned to the venous reservoir via the cardiotomy suction), and thus was detectable in high quantities in the peripheral blood, did so due to a cross-reactivity of S100β with several other high molecular weight proteins. As a result, although S100β is detectable using the commercially available ELISA, proteins other than S100β were actually being detected.

This new finding, however, does not fundamentally change the fact that although there may be a relationship—albeit weak—between early S100β elevations and neurologic injury, the relationship is, at best, an indirect

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